XX.—The Phase of the Nucleus known as Synapsis.

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(With Two Plates.)

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That phase of the nucleus just preceding the heterotype mitosis, and which was first called *Synapsis* by MOORE (1895), has come to be regarded as an important and critical period in the life-cycle. It was called synapsis because of the apparent condensation and contraction of the chromatin at one side of the nuclear cavity. This discovery at first received scant support, the *synaptic contraction* being considered by many investigators to be nothing more than an artifact—an artificial contraction caused by imperfect fixation. In 1897, however, MOORE'S discovery was confirmed by Miss SARGANT, who not only observed it in fixed material, but also found it in the living pollen-mother-cells of *Lilium*.

Since that time the importance of this apparently contracted condition of the chromatin has steadily grown with the increase in our knowledge on the subject of reduction division, so that many of the eminent and leading cytologists of the day have come to interpret this phase known as synapsis as that critical period when the actual fusion and consequent reduction of the chromosomes take place. It is believed by some to represent the actual blending of the maternal and paternal chromatin. As one writer expresses it, "It is the critical stage in the history of an organism. It is the end-result of fertilisation."

That such a *stage* exists, I think, there can be no question. It has been observed and described by too many careful and eminent workers to warrant a denial of its existence. These observations not only come from every botanical centre where cytological investigation is carried on, but they have been made upon representative types of all the main groups in the plant kingdom.

In her work on *Lilium*, Miss SARGANT (1897) tells us that "the approach of synapsis is first indicated by the appearance of drops of nuclear matter adhering to the chromatic network. . . . A little later the nucleoli lose their well-defined outline, the nuclear membrane becomes indistinct, and the chromatic threads show a tendency to collect round the nucleoli at one side of the nuclear cavity. . . . The nucleus of the pollen-mother-cell has now entered on the period of contraction called synapsis which precedes the formation of the spireme threads."

In ALLEN'S (1904) account of this stage in *Lilium canadense* we find the statement that "the fusion of the threads and of the chromosomes occurs very early in synapsis; but after the fusion the synaptic condition persists, certainly for days, perhaps for weeks or more. Towards the end of this period, the aggregation of the spireme becomes gradually looser, and then follows a stage in which the thread is very evenly distri-

buted throughout the nuclear cavity and is in contact with the nuclear membrane at very many points."

FARMER and MOORE (1905), in their description of this stage in *Lilium*, state that "at first irregularly coiled in the nucleus, the differentiating spireme next aggregates towards one side, and there forms what we may designate as 'the first contraction figure.' The thread becomes densely coiled in the vicinity of the nucleolus, exhibiting a highly characteristic arrangement. This figure has often been dismissed as the result of imperfect fixation, but there exists strong evidence to show that it represents a normal occurrence in the life-history of the cells. . . . It is a stage that persists for some time; but, as it passes away, the filaments become loosely coiled and diffused, especially about the periphery of the nuclear cavity. It is perhaps a fact of some significance that the nucleus at this stage is relatively large, the average diameter in the case of pollen-mother-cells of *Lilium canadense* being 32 μ, as compared with the diameter 29 μ reached by the nuclei at the contraction figure just described."

In his endeavour to explain the lateral position of the "*synaptic knot*," CARDIFF (1906) states that this is probably due to gravity. "In fact, the knot seems to be as often, if not more often, on the side of the cell where there is least cytoplasm. It was generally found, however, that in any one sporangium or group of sporangia all the knots occupy the same relative position in the nuclei. I offer as a tentative explanation of this, that the chromatin mass is of greater density than the nuclear sap and the position of the nucleolus and knot is due to gravity."

GATES (1907) describes the synaptic contraction in the hybrids of *Œnothera* as follows: "The spireme gradually contracts into a dense ball with a few loose threads projecting irregularly. In this closely contracted condition it may form a body about the size of the nucleolus, which can only be distinguished from the latter by its somewhat irregular outline. In the next stage observed the spireme is again loosely arranged in the nuclear cavity, but is greatly contracted in length and several times in thickness of the original spireme before the contraction stage."

MOTTIER'S (1907) account of synapsis is given more in detail: "With the rapid increase in size of the cell and nucleus, the entire contents contract or ball up towards one side of the nucleus. This contraction is a rapid process, and whether the rapid growth of the nucleus is a stimulus to this contraction is an interesting question. The nucleolus may be included partly or wholly within the contracted mass, or it may lie merely in contact with it, or entirely free at a remote side of the nucleus. As a rule the nucleolus is included within the chromatin mass. When complete synapsis is reached the mass is tightly balled up, many nuclei showing no free portion projecting into the nuclear cavity. There seems to be little or no regularity in the position of the synaptic mass in the nucleus as regards the upper or lower ends or the sides. CARDIFF states that in the plants studied by him gravity determines the position which the mass shall take. This explanation does not hold for *Podophyllum* or *Lilium*. . . . In the contracted mass no definite structure can be made out. Sometimes the appear-

ance is that of a balled-up mass of lumps or granules, and sometimes that of chromatin thread."

Among the Fungi, synapsis has been found by Miss FRASER (1908). She describes it in her account of *Humaria*: "The stainable material of the nucleus forms a fine threadwork and becomes aggregated towards one side of the nuclear membrane, forming the first contraction figure, as has been described for the spore-mother-cells of the higher plants."

For the Ferns we have an account of synapsis in *Nephrodium* by YAMANOUCHI (1908). His account is as follows: "As described before, the nucleus in the pre-synaptic stage consists of a complex, anastomosing, chromatin reticulum. This ragged reticulum shows a tendency to become transformed into a thread structure, but the process does not occur simultaneously in the different regions. When the transformation has taken place, the two parts of the thread are observed running side by side from the first. Such a condition, etc., is evidently what was called 'leptonema' by WINIWARTER. Further transformation of the thread structure from the ragged reticulum results in a nucleus with a continuous chromatin thread in a spireme whose double nature is only visible at certain parts on account of the close association. The thread becomes tangled and contracted in one side of the nuclear cavity until finally there results the climax of the synaptic stage. . . . There are observed in *Nephrodium* a number of parts of the spireme running through the contracted mass from the nuclear membrane where the mass lies in contact."

Even in the more recent literature the contracted condition of the chromatin is fully described and its relation to the reduction process is emphasised. In his work on the organisation of the nuclei in pollen-mother-cells, OVERTON (1909) states that the first indication of synaptic contraction in *Thalictrum purpurescens* consists in a concentration of the paired spireme threads either in the centre or at one side of the nuclear cavity, so that the nucleus appears much more open and clear than at earlier stages. "During the earlier stages of this contraction the threads which constitute the framework are present, but then later disappear as the whole mass rounds up. In my former paper I have described the phenomenon of synapsis, which I held to be a normal process, in which a union in pairs of homologous elements is brought about with a consequent pseudo-reduction. Although synapsis was described as a phase, during which a mutual interchange or interaction of parental elements might occur, I also expressed the opinion that the homologous chromosomes retain their identity during the process."

In his work on *Œnothera*, DAVIS (1909) defines synapsis as a "general slow contraction of the reticulum away from the nuclear membrane, a contraction that carries most of the strands towards the centre of the nucleus. During the process of contraction numerous threads are differentiated from the reticulum, which become coiled in a very intricate manner." DAVIS further describes how "the synaptic contraction draws the coils of threads into a dense knot close to the large nucleolus, which generally lies at one side of the nucleus. Loops of the threads extend into the nuclear cavity from the

synaptic knot as a centre. The threads gradually thicken as synapsis proceeds, and the length of the thread system is very much shortened. When fully contracted the synaptic knot consists of much-thickened threads (constituting the spireme) which are drawn so tightly together that their arrangement cannot be traced. The loops extending from the contracted mass are at this stage thick and very conspicuous."

STRASBURGER, MIYAKE, GRÉGOIRE, BERGHS, and others have expressed similar interpretations of this phenomenon, but the above extracts are sufficient to show how the idea of synapsis has grown.

Now, it is not the purpose of the present work to deny the existence of this *stage* known as synapsis. I have observed it in many representative types of Algæ, Fungi, Bryophyta, Pteridophyta, Gymnosperms, and Angiosperms, and I am quite convinced that it is a constant and normal phase in the nuclear-cycle. My interpretation of this phase, however, is not in agreement with any of the above-quoted writers or with any other interpretation which to my knowledge has yet been published. After a careful study of many types, I have been convinced that during this phase known as synapsis there is no contraction whatever of the chromatin substance; that the condition so often described as "contracted" is not in reality a contraction, but is subject to quite a different interpretation. I have also been convinced that this so-called "contraction stage" has nothing whatever to do with the blending or fusion of maternal and paternal chromatin and consequently plays no immediate rôle in the process of chromosome reduction.

As these interpretations are so widely opposed to those of so many eminent cytologists, I have delayed in publishing them until they had been confirmed by a study of a wide range of forms extending from the Algæ to the Angiosperms. This has been done, and from the observations made I have no longer any hesitation in stating my views.

Now, while the following observations are confined to the microspore-mother-cells of a single Angiosperm type, *Smilacina*, this was done for the convenience that this particular plant afforded in obtaining an unbroken series of stages of nuclear changes during the heterotype mitosis. All of the main conclusions arrived at from a study of this plant were later confirmed by an investigation of types from the Gymnosperms, Pteridophyta, Bryophyta, and Algæ. *Smilacina* is a particularly favourable plant for such a study, for the flowers are quite small and very numerous in a close inflorescence. The entire young inflorescences were taken and fixed in chrom-acetic acid without dissection. After being imbedded in paraffin and sectioned, it was found that while at the base of the raceme the tetrads were fully formed, at the apex the Archesporium was not yet organised and that between these two extremes every conceivable stage of nuclear activity was to be found. The advantages of having so many stages in a single section are obvious—they allowed of a close and accurate comparative study of the different stages in their natural sequence.

The mature Archesporium showed the mother-cells fitting closely to one another with the walls very thin and in the form of straight lines and sharp angles—leaving no room

whatever for intercellular spaces. The cytoplasm was characteristically sporagenous in being densely charged with fine food granules and containing no vacuoles of measurable size. The nucleus was practically spherical in form and nearly centrally situated (fig. 1). There were two or sometimes three large nucleoli present. The chromatin with its linin appeared as a fine mesh or network of threads. This network, however, is more apparent than real, for, by carefully focusing, one could follow the individual threads passing over and interlacing with one another for considerable distances. The individual threads were finely granular, and, on account of their apparently anastomosing with one another, it was impossible to count or even approximately estimate whether they corresponded to the diploid number of chromosomes. Apart from observing that there were a number of threads of chromatin present, I was unable to distinguish "prochromosomes," which have been recorded as occurring in certain Dicotyledons by OVERTON (1905) and others.

The almost spherical form of the nucleus as well as its large size—as compared with that of the vegetative cells—gives the impression that the nuclear sap is exerting a considerable osmotic pressure upon the nuclear membrane. In this connection it should be remembered that in the cytoplasm there are no vacuoles in the sense that we have them in the growing vegetative cells, the entire cytoplasm being charged with food substances, and yet these spore-mother-cells are actually growing in size. This circumstance, together with the turgid appearance of the nucleus, makes it difficult to escape from the conclusion that the nuclear cavity is acting as a vacuole. That the enlargement of the nuclear cavity is brought about by the increase in the amount of its contained fluid there can be no doubt, and an increase of osmotic pressure acting upon the nuclear membrane would necessarily follow. This finds an expression in the stretching of the nuclear membrane and a considerable increase of the nuclear space. The nuclear cavity thus acts as a vacuole in exerting a great internal osmotic pressure, and, in doing so, facilitates growth in a cell where ordinary vacuoles are absent from the cytoplasm. This seems a plausible explanation for the great size of the nuclear cavity of spore-mother-cells. And, as we shall point out, the growth of these cells proceeds with the increase in the size of the nucleus.

In fig. 2 we have represented a stage where the increased amount of nuclear sap shows itself by a slight distension of the nuclear membrane beyond the area occupied by the chromatin. On the lower side of the figure one sees a clear space—appearing as a narrow crescent in section—between the chromatin and the membrane. This, at first sight, might be mistaken for a shrinkage of the chromatin, but the comparatively even and regular surface of the chromatin area, as well as a consideration of the relative size of the latter in the preceding fig., makes it quite evident that the clear crescent-shaped area is due not to shrinkage or contraction, but to an increase in the nuclear sap with the consequent distension and withdrawal of the nuclear membrane from the chromatin.

In fig. 3 is represented a slightly later stage, where the distension of the membrane

and its removal from the chromatin is more obvious, with its much larger clear area of nuclear sap. This clear area of nuclear sap generally made its appearance at one side especially in the earlier stages; but this was not always the case. Nuclei were sometimes found with the clear nuclear sap completely surrounding the chromatin, as indicated in fig. 4. This difference one would naturally expect, under the circumstances; for with so many growing cells closely packed together, the resistance offered to the increased osmotic pressures within the nuclei would not be constant and uniform for each cell. Where such resistance was practically uniform we would expect to find the condition shown in fig. 4; but where the resistance was not uniform on all sides, the conditions shown in figs. 2, 3, 5, etc., would result, because the growing nucleus would naturally distend in the direction of least resistance. In fig. 4 the distension of the nuclear cavity, while not uniform in all directions, is nearly so. In fig. 5 we see the distension is very marked on one side and very slight on the other. Similar conditions are shown in figs. 6, 7, and 8, while in fig. 9 the distension must have been nearly uniform in all directions, resulting in an almost perfectly spherical form of the nuclear cavity with a spherical mass of chromatin in the centre.

Now, any of these stages, if examined individually and not in series, might easily be mistaken for an early stage of what is known as the *synaptic contraction*. But when compared with one another in their natural sequence, it becomes quite clear that no contraction whatever has taken place in the chromatin mass. This can easily be demonstrated by measuring the chromatin area of the various stages figured. Such measurements have been taken in a great number of cases, and no differences could be detected in the size of the chromatin masses.

In figs. 10, 11, 12, and 13 we show a series of later stages, any one of which corresponds to what has been described by other writers as *synapsis*, *synaptic knot*, or *violent contraction of the chromatin*, etc. It is quite obvious that these figures represent merely a continuation of the process described above for the earlier stages—namely, a gradual and further accumulation of nuclear sap, an enlargement of the nuclear cavity, and a still further withdrawal of the nuclear membrane from the chromatin. Several writers, in describing this stage, state that "the chromatin moves to one side of the nuclear cavity and there coils up into a tight ball." The general appearance of any one of these stages certainly suggests such an interpretation; but, upon comparing them with the preceding and following stages, no real evidence can be found to support it. Indeed, all of the facts which I have been able to obtain from my own preparations go to prove the opposite—namely, that the chromatin mass neither moves to one side nor does it contract.

Of the numerous figures of synapsis that have been published in recent papers, many of them show the chromatin mass with a much smaller area in proportion to the nuclear cavity than I have here figured for *Smilacina*, which would indicate a certain amount of contraction. I have also found much smaller areas of chromatin at this time and might have figured them, but they were rejected. I attributed them to either

imperfect fixation or to the fact that the sections were not cut in a median plane through the nucleus. It should perhaps be pointed out that the larger the nuclear cavity becomes, the more osmotically sensitive become the substances within the limiting osmotic membrane. Consequently this stage offers perhaps more difficulties for proper fixation than any other stage in the history of the nucleus. This was made manifest in several lots of material which were fixed in Fleming's strong solution. It was found that whenever this strong osmic acid solution was used for fixing, the cytoplasm appeared more or less plasmolysed and the chromatin mass within the nuclear membrane showed a corresponding amount of shrinkage. In this same material, however, the early stages of the mother-cells—before the increased size of the nuclear cavity—showed perfect fixation. It would therefore seem that the very much enlarged nuclear cavity favours a shrinkage of the cytoplasm as well as of the chromatin, and this condition of the latter might easily be mistaken for a natural contraction. In working out the present series every slide was rejected that showed the slightest trace of plasmolysis.

As stated above, certain sections were passed over because they were not cut in a median plane through the chromatin mass. In this connection it might be well to point out that when the nucleus reaches its full size it may be 25 μ or 30 μ in its longest diameter, and, cutting sections of an anther at this time, we invariably obtain numbers of sections of cells that do not pass through the median line of the nucleus. Some of these sections may show a very large nuclear cavity and a very small shaving of chromatin at one end. Such small areas of chromatin at first suggest a considerable contraction, and they were frequently found. This fact is mentioned here because I feel sure that some of the figures that have been published to show the synaptic contraction have been drawn from such oblique sections. Fig. 13 is inserted here to illustrate this point. It was drawn from a section that was not median. All of the other figures were drawn from sections that were cut in a median plane—or nearly so—through the chromatin mass. Now, if we examine all of these stages from figs. 1 to 12 in series, we find no diminution whatever in the area occupied by the chromatin. At the same time it is obvious that the nuclear cavity has increased to nearly twice its original size. As we show in figs. 16, 17, 18, 19, and 20, this enlargement of the nuclear cavity gradually continues until it reaches a cubical dimension which is quite three times that shown in fig. 1. But during this entire period of growth there is not the slightest evidence that the chromatin mass has shrunken or contracted. The obvious conclusion I draw from this is that, owing to the greater osmotic pressure caused by the increase of the karyolymph, the nuclear membrane has distended and withdrawn from the chromatin mass. The latter remains in its original position and has not moved away from the membrane, as has so frequently been stated by so many writers.

Now, while no actual contraction could be detected in the area occupied by the chromatin during this period of growth of the nucleus, the threads of the chromatin undergo a distinct modification. In the very early condition shown in fig. 1 the chromatin appears as a distinct but irregular network. This network or mesh is com-

posed of extremely delicate threads that could be followed for short distances; but as they appear to interlace and anastomose with one another, it was impossible to determine their length or their structure. In the next stages, figs. 2, 3, and 4, where the first indication of the distension of the nuclear membrane was noted, we find the chromatin threads slightly thicker and evidently granular and the interstices between them larger and more clear. This would suggest a shortening and thickening of the threads, but not a contraction. These changes continue as indicated in figs. 5, 6, 7, and 8, and they appear to progress with the growth of the nuclear cavity. In these figures a very interesting and important fact is revealed—namely, that the chromatin threads are undoubtedly double. If these stages were examined individually, one might interpret this as a longitudinal splitting of the thread at this early period; but, by tracing the series back to fig. 1, I was unable to pick out a stage where there was not evidence of the double nature. This series would certainly suggest that the chromatin threads were double from the beginning, but the double nature is only revealed with certainty after the stages represented in figs. 5, 6, and 7 have been reached. In figs. 9 and 10 we find the chromatin threads have become very sharply defined and may be followed for considerable distances. Not only is the double nature of the threads more easily made out, but the ends of the threads could be seen. Some of these ends are, no doubt, due to sectioning, and as such appear on the surface. Many of them, however, were undoubtedly within the interior of the mass, and these I take to be the actual ends of the chromatin threads. As many of these ends were found—the double nature of which could be made out,—this fact would indicate that the developing spireme was not composed of a single continuous thread, but of a number of double threads. In tracing the series back, I could not determine when the ends first became visible in the earlier stages; the threads being so delicate, one could not say positively whether one was examining the granules on the reticulum or the thread ends. The inference I draw, however, from these observations is that the reticulum, represented in fig. 1, is made up *of a definite number of threads* which are double, and that this number corresponds to the diploid number of chromosomes which become *differentiated later.*

In figs. 11, 12, 13, and 14 we have a much better opportunity of studying the double nature of the threads, for we now find them projecting out at intervals from the main mass into the large clear space occupied by the nuclear sap or karyolymph. In fig. 11 we see the beginning of a general loosening and separation of the threads from one another. This loosening of the spireme seems to be a gradual, but irregular, process. As indicated in figs. 10, 11, and 14, the ends of the threads project for a short distance into the main body of karyolymph, but, as may be seen in figs. 12, 13, 15, 16, and 17, they soon extend out for a great length—some of them reaching the nuclear membrane at the opposite side. In such cases the double nature of the threads may be observed without difficulty.

During this process of loosening and spreading out of the chromatin into the clear area of the nuclear cavity the threads have continued their process of shortening and

thickening, and this is brought out in figs. 18, 19, 20, and 21; but they never lose the evidence of their double nature. In most cases at this time the double threads showed the presence of disks or short segments—the chromomeres (ALLAN, 1905). These are indicated quite clearly in figs. 19, 22, 23, and 24.

In the stages represented by figs. 25, 26, and 27, the threads of the spireme have become so much differentiated that they are easily identified as definite chromosomes. They have become so much separated from one another that they appear evenly distributed throughout the very much enlarged nuclear cavity. Each chromosome may be followed from end to end without difficulty.

There are, then, during this growth period of the nucleus, certain interesting and important changes in the nature of the chromatin threads, but I find no evidence whatever that the chromatin has contracted. My interpretation of synapsis is simply that it represents a "growth period" of the nucleus—a period during which there is a great increase in the amount of nuclear sap which results in a distention and withdrawal of the nuclear membrane from the chromatin. The question that naturally arises is, Why should this growth period occur in the life-history only at a time immediately preceding the reduction division? My answer to this is suggested in certain statements made in the early part of this paper in regard to the contents of these mother-cells. Each one of these cells is charged with sufficient food substance for the production and sustenance of four spores. In being so charged they are really storage cells—storage cells that have the power of merismatic activity. Moreover, this merismatic activity is not of the ordinary kind, for it finds an expression in two divisions that follow one another very rapidly. Then, again, these cells exhibit a marked power for rapid growth. Now, nowhere else in the life-history do we find cells with such active and varied properties. It is not surprising, therefore, that we find they differ in their constitution from ordinary vegetative cells. One striking difference is the absence of vacuoles from the cytoplasm. In ordinary growing vegetative cells there is a great production of cell-sap. This accumulates in the vacuoles and generates an osmotic pressure which facilitates growth. In these growing spore-mother-cells, on the other hand—which are both storage and merismatic in character—there are no vacuoles of any measurable size, and consequently there are no open bodies of cell-sap accumulated in the cytoplasm. There is, however, a great body of sap accumulated within the nucleus, which gives the latter its characteristic appearance and distinguishes it at once from ordinary vegetative nuclei. It is, of course, out of the question to prove by actual experiment that the enlarged nuclear cavity produces an internal osmotic pressure, and distends the cell in the same manner that the vacuole is believed to do in the case of vegetative cells. The distended and turgid condition of the nucleus during this period of growth, in addition to its great size, is, however, sufficiently convincing that the pressure set up by the nuclear cavity is just as great as that set up by a vacuole of like dimensions. It is quite common, even among vegetative cells that have been especially differentiated for storage or secretion, to find the nuclear

cavity very much enlarged, but not perhaps to the extent of these mother-cells. These latter cells have an important and varied series of functions to perform, and their organisation is modified accordingly. Not the least of these modifications I believe to be the taking on of the function of the vacuole by the nuclear cavity. While this cannot be actually proved, I think the evidence is sufficient to warrant my offering it as an explanation for the occurrence of the "growth period" of the nucleus at this particular phase in the life-history—a phase that is commonly known as synapsis.

The majority of writers on the subject have made much of the lateral position of the chromatin mass within the nuclear cavity during the "synaptic period." The mass is invariably described as occupying a position at one side of the cavity, close to the nuclear membrane. This position, I think, is responsible in a large measure for the contraction idea, for it certainly gives one the impression of a shrinkage or withdrawal from the membrane. More than one writer has described the chromatin as having moved bodily from one side of the cavity to the other—CARDIFF (1905) even goes so far as to state that this lateral position is due to gravity. If there had been a "violent contraction," as one writer describes it, the lateral position would seem obvious enough. If, however, there is no contraction whatever, there must be some reason for this peculiar and characteristic condition.

I have above called attention to the fact that the young spore-mother-cells, composing the Archesporium, lie closely together, forming a dense mass of tissue with very thin walls and no trace of intercellular spaces. The straight lines and sharp angles that mark the boundaries of the cells are shown in figs. 1, 2, 3, 4, and 5. As these cells grow in size, there is also a growth taking place in the anther as a whole, and, as development advances, there is more room within the sporangium for the mother-cells to enlarge. Now, these cells not only enlarge but, as everyone knows, they later separate from one another and eventually lie free in the mother liquor of the anther. This separation of the mother-cells from one another first manifests itself by the presence of small intercellular spaces. After an examination of many sections I find these spaces occur more frequently at the angles where two or more cells meet. At such points the thin cell-wall becomes rounded off (figs. 15, 17, and 18), clearly indicating that the internal pressure was exerting itself towards the intercellular space. This rounding off of the angles of the cell-wall proceeds exactly with the development of the intercellular spaces, until we finally have the rounded or oval shapes of the cells as shown in the figs. 20, 21, 22, 23, 24, etc. It is quite clear from these figures that the growth of the cells has taken place in a direction towards the first intercellular space that is formed. Nearly all of the cells become more or less oval in outline, but one end is always more rounded than the other. The latter end invariably shows the wall in straight lines and angles, indicating contact and pressure against its neighbours (figs. 22, 23, and 24). The rounded end is always free and exposed to the intercellular space. Now, if we examine any of these stages that are figured, we will find that the enlarged nuclear cavity is always extended towards the rounded end of the cell. It will also be found that in

none of these growing stages is the nuclear cavity spherical in form. It is invariably oval or egg-shaped. As soon, however, as the mother-cells are practically free from one another, as illustrated in figs. 26 and 27, the nuclear cavity assumes an almost spherical form. Here the internal pressure is apparently exerting itself equally in all directions. These facts not only show that a great internal pressure exists within the nuclear cavity, but also that the effect of such a pressure on the resultant growth and form of the nuclear cavity is controlled by surrounding resistance. This being the case, the internal pressure would naturally exert itself in the line of least resistance, and that would be towards the intercellular space. The conditions shown in figs. 20, 21, 22, and 23, would seem to indicate that this is exactly what has taken place. In this I see a reasonable explanation for the lateral position of the chromatin mass during the growth period. It is simply the result of the enlarging nuclear cavity extending out towards the intercellular space where there is least pressure from the neighbouring cells and leaving the chromatin mass behind in its original position. Considering all of the circumstances under which the mother-cells are developing, the lateral position of the chromatin mass during the growth period is a perfectly natural one.

The object of the present paper is simply to state my interpretation of that phase of the nuclear cycle commonly known as synapsis and also the reasons for such interpretation. I do not intend in this work to go into the details of reduction—this will be published shortly in a separate paper. I may state, however, that I have strong evidence for believing that the reduction process cannot take place in *Smilacina* during the growth period. In figs. 26 and 27 we have represented stages in the development of the mother-cells which are undoubtedly much later than that known as synapsis. Here we have a condition where the chromosomes may be observed with sufficient clearness that they may be counted. After repeated counting I estimated that the number at this time is twenty, which is just twice the number found in the haploid phase. The real act of reduction is shown in fig. 28, which is at a time much later than that shown in fig. 27.

Summary.

Spore-mother-cells, being both storage and merismatic in their function, present an organisation that is strikingly different from that found in ordinary vegetative tissue.

Being charged with food substances for the production and sustenance of four spores, they are devoid of vacuoles of any measurable size in the cytoplasm.

During their development, however, there is a great accumulation of sap within the nuclear cavity, which causes a great osmotic pressure in the same manner that the cell-sap does in the vacuole of growing vegetative cells.

The pressure, acting from within, causes the nuclear membrane to distend and the nuclear cavity to expand.

This expansion, at first gradual, continues until the nuclear cavity grows to twice or even three times its original size.

As this growth proceeds the membrane is gradually withdrawn from the chromatin mass within.

The result of this withdrawal of the nuclear membrane is the formation of a large clear area of nuclear sap containing the mass of chromatin which has been left at one side.

No evidence whatever was found to show that any contraction of the chromatin had taken place.

The enlargement of the nuclear cavity and the consequent withdrawal of the membrane away from the chromatin give the appearance of a contraction, but actual measurements failed to show any diminution in the chromatin area.

Although no contraction takes place during the growth period of the nucleus, certain definite and important changes take place in the nature of the chromatin threads as the spireme becomes differentiated.

In tracing the series of developmental stages back, there was some evidence that the reticulum was composed of a number of threads and that this number corresponds with the diploid number of chromosomes.

As the chromatin passes from the reticulum to the spireme condition, there was no stage found that did not show some evidence of the double nature of the threads. The inference drawn from this is that the chromatin threads are double, even in the reticulum stage.

During the entire growth of the nuclear cavity there is a gradual shortening and thickening of the chromatin threads until they become differentiated into definite chromosomes, but in no case was there any evidence of a blending or fusion of these bodies.

It was further found that the actual reduction occurs at a time much later than that commonly known as synapsis.

My interpretation of the phenomenon known as synapsis is simply that it represents a growth period of the nucleus—a condition that is in harmony with the peculiar organisation of spore-mother-cells. It is a period during which the increasing karyolymph exerts a great osmotic pressure from within. This pressure results in the extension of the nuclear cavity towards an intercellular space where there is least resistance from the neighbouring cells. The chromatin mass is left behind, and its characteristic position at one side of the nuclear membrane is a perfectly natural one.

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EXPLANATION OF FIGURES IN PLATES.

All the figures were drawn with the camera lucida to the same scale with Zeiss compensating ocular, No. 8, and Zeiss oil imm. obj. 1/12. × 1600.

Fig. 1. A young spore-mother-cell with the nucleus in the resting stage. The chromatin is in the form of a reticulum composed of very delicate threads.

Fig. 2. A slightly older stage of the same, showing the first indication of the distension of the nuclear membrane and its withdrawal from the chromatin mass.

Fig. 3. The same, still older, but here, in addition to the further distension of the nuclear membrane, the chromatin threads of the reticulum are becoming thicker and more sharply defined.

Fig. 4. The same. An example of the withdrawal of the membrane from all sides of the chromatin mass.

Fig. 5. The same, showing a further distension of the membrane.

Fig. 6. The same at a later stage, with a marked change in the development of the chromatin threads.

Fig. 7. The reticulum has taken the form of a definite spireme.

Fig. 8. A further withdrawal of the nuclear membrane from the chromatin. The spireme is now sharply defined. The ends of the threads may be seen, as well as the double structure.

Fig. 9. The same. The pressure within the nuclear cavity is evidently exerting itself equally in all directions.

Fig. 10. The nuclear cavity has enlarged to nearly twice its original size, but there is no evidence of contraction of the chromatin.

Fig. 11. The nuclear cavity has enlarged still more. The threads of the spireme are beginning to loosen and project into the clear area of the nuclear sap. The double nature of the threads may be clearly seen.

Fig. 12. The same, but a later condition of the loosening of the spireme threads.

Fig. 13. The same. The nuclear cavity has enlarged still more.

Fig. 14. The same, from a section that has not been cut in a median plane through the chromatin mass.

Fig. 15. A further stage in the development of the spireme. The double threads are much more sharply defined and their ends are clearly visible.

Fig. 16. The nuclear cavity is now more than twice its original size.

Fig. 17. The rounding off of the corners of the mother-cells indicating the presence of an intercellular space. The distension of the nuclear cavity is directed towards this intercellular space, leaving the chromatin behind at the opposite side.

Fig. 18. A more striking example of the same.

Fig. 19. The spireme threads now show the short segments or "chromomeres."

Fig. 20. This stage is an excellent example to show the distension of growing nuclear cavity towards the intercellular space. That is, it extends in the line of least resistance leaving the spireme behind.

Fig. 21. The loosening of the spireme becomes more evident.

Fig. 22. Another stage of the same.

Fig. 23. The spireme threads projecting and extending into the large clear area of the nuclear sap.

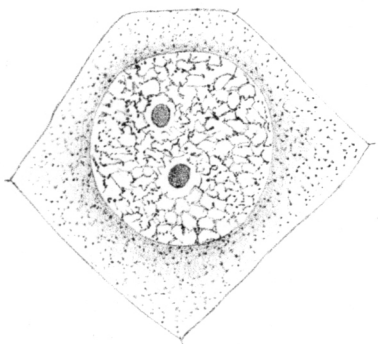
Fig. 24. Another stage of the same.

Fig. 25. The spireme threads are now nearly uniformly distributed through the enlarged nuclear cavity.

Fig. 26. The chromosomes may now be identified and followed from end to end. The enlarged nuclear cavity has resumed its spherical form owing to the fact that the mother-cells are practically free from one another.

Fig. 27. This stage clearly shows the diploid number of chromosomes, proving that reduction could not possibly have taken place in any of the previous stages.

Fig. 28. A much later stage, showing the chromosomes consorting in pairs.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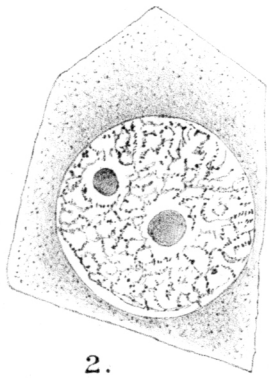
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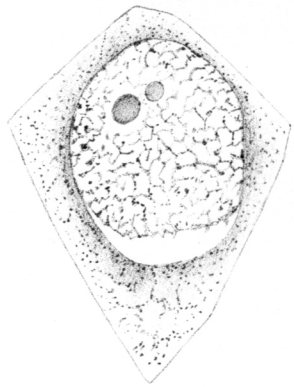
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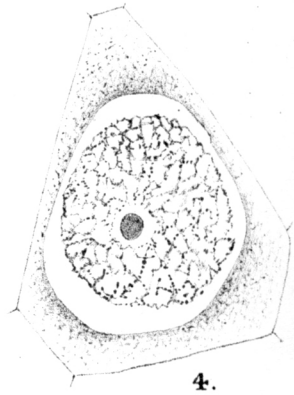
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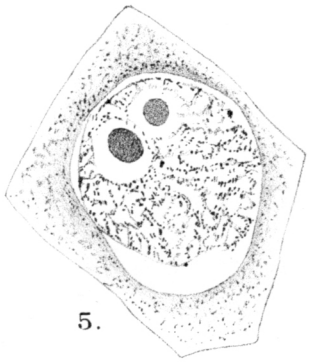
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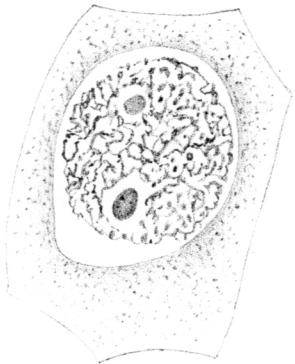
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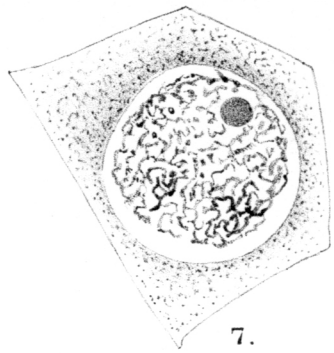
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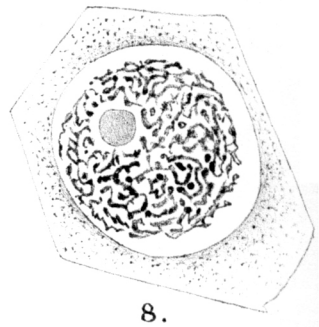
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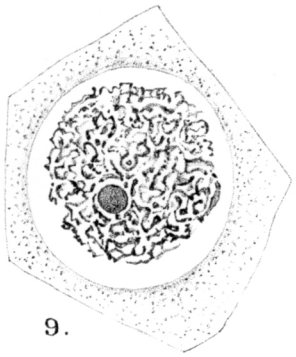
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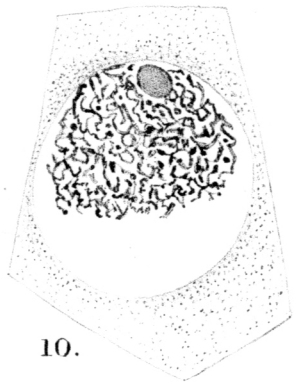
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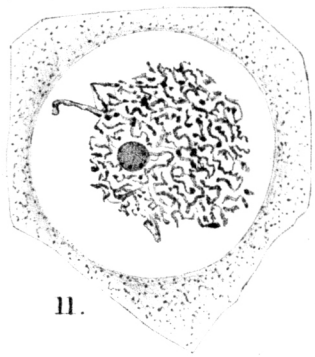
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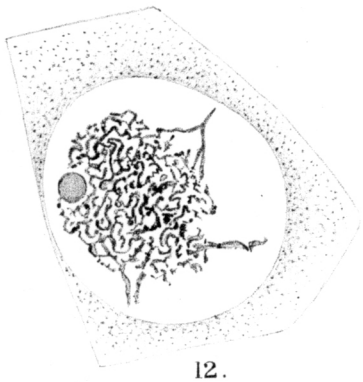
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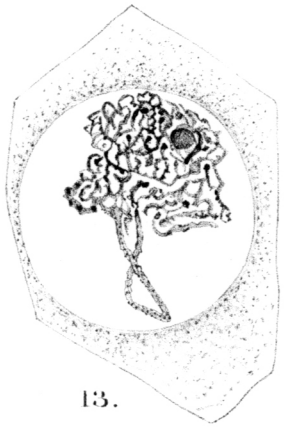
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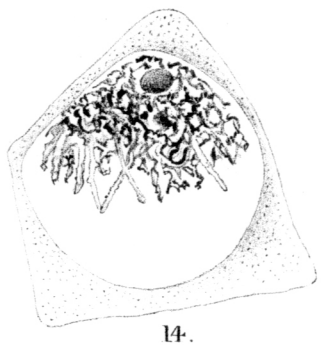
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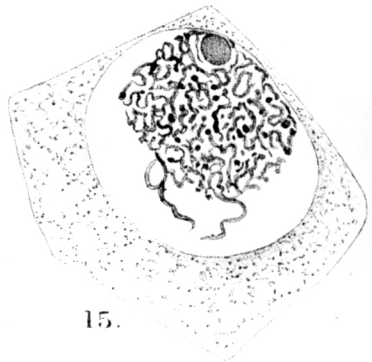
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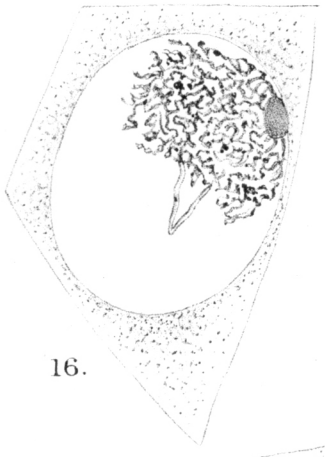

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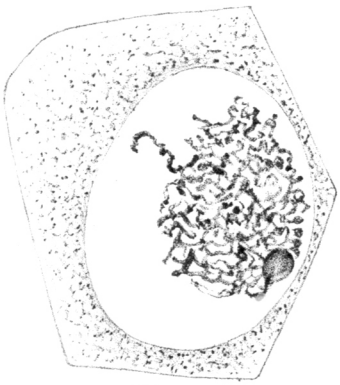

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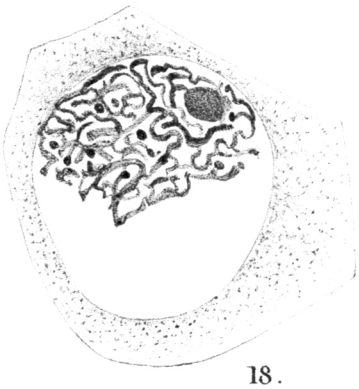

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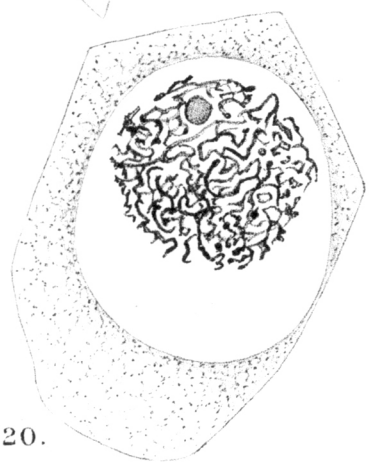

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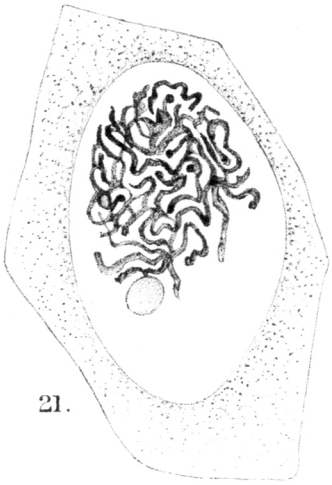

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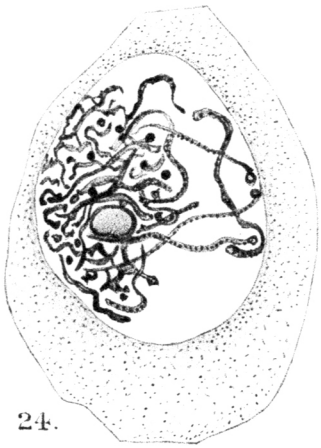

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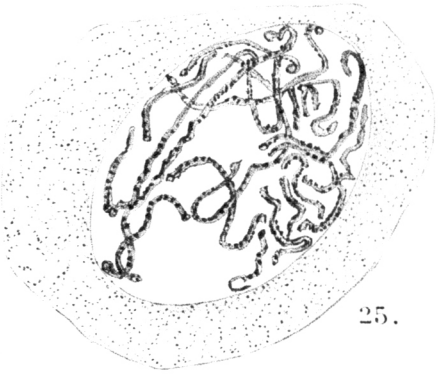

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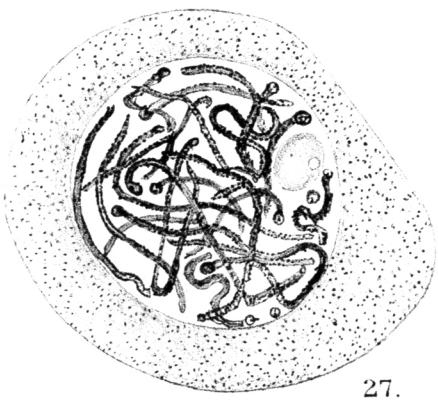

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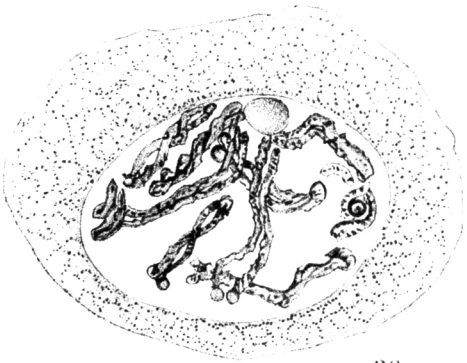

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